REMARKS

The indication by the Examiner in Item 5 on page 2 of the Office Action mailed July 6, 2010, is noted. As appreciated by the Examiner, there was an error in setting forth the application number in the header on each of pages 2-25 of the Amendment filed April 12, 2010. Such error is regretted. The application number in the heading on page 1 of the Amendment filed April 12, 2010, was correct, and is now correct in the header on pages 2-25.

Applicants are amending their claims in order to further clarify the definition of various aspects of the present invention. Specifically, Applicants have amended claim 1 to recite that the probing with a scanning probe microscope is performed in a solution "which is an aqueous solution containing a salt, or a buffer solution". Note, for example, page 16, lines 3-8, of Applicants' specification.

In addition, Applicants are adding new claim 35 to the application. Claim 35, dependent on claim 19, recites that the solution is an aqueous solution containing a salt, or a buffer solution. In connection therewith, attention is respectfully directed to page 16, lines 3-8, of Applicants' specification.

The concurrently filed RCE Transmittal is noted. It is respectfully submitted that the present amendments, and following Remarks, constitute the necessary Submission for this RCE Transmittal; and it is respectfully submitted that in view of filing this RCE Transmittal, entry of the present amendments is clearly proper, notwithstanding the Finality of the Office Action mailed July 6, 2010.

Applicants respectfully submit that all of the claims presented for consideration by the Examiner patentably distinguish over the teachings of the documents applied by the Examiner in rejecting claims in the Office Action mailed July 6, 2010, that is, the teachings of the U.S. patent documents to Henderson, et

al., Patent Application Publication No. 2002/0042081 (hereinafter, consistent with the Office Action mailed July 6, 2010, referred to as "Henderson-2, et al."), to Peeters, Patent No. 6,762,056, and to Obremski, et al., Patent Application Publication No. 2002/0001853, and the article by Liu, et al., "Production of Nanostructures of DNA on Surfaces", in Nano Letters (2002), Vol. 2, No. 8, pp. 863-867, under the provisions of 35 USC 102 and USC 103.

It is respectfully submitted that the teachings of the applied documents would have neither disclosed nor would have suggested such a molecular detection method as in the present claims, comprising visualizing and identifying an individual chain molecule immobilized on a plastic substrate surface and as immobilized being uprightly disposed, by probing with a scanning probe microscope in a solution so as to observe a profile of the plastic substrate surface having individual chain molecules immobilized thereon and observe the individual chain molecules immobilized uprightly on the plastic substrate surface, wherein the solution in which the probing is performed is an aqueous solution containing a salt, or a buffer solution (see claim 1; note also claim 35), and/or wherein the chain molecule immobilized on the plastic substrate surface is a nucleic acid (see claim 19).

Moreover, it is respectfully submitted that the teachings of these documents as applied by the Examiner would have neither disclosed nor would have suggested such a molecular detection method as in the present claims, having features as discussed previously in connection with claim 1, including the visualizing and identifying by probing with a scanning probe microscope in the specified solution so as to, inter alia, observe the individual chain molecule immobilized uprightly on the plastic substrate surface, especially wherein the profile is observed using an atomic force acting between the plastic substrate surface having the individual chain

molecule immobilized thereon and a probe of the scanning probe microscope (see claims 29 and 33), particularly wherein the profile is observed by measuring an amount of flexing of the probe caused by the atomic force (see claims 30 and 34).

Additionally, it is respectfully submitted that the teachings of the applied documents would have neither disclosed nor would have suggested such molecular counting method as in the present claims, including detecting a molecule by the method of claims 1 and 19, and counting the number of detected chain molecules per unit area (see claims 6, 7, 23 and 24); or the molecular localization detection method as in the present claims, wherein counting of the number of detected chain molecules per unit area gives molecular localization information (see claims 7 and 24).

Furthermore, it is respectfully submitted that the teachings of these applied documents would have neither disclosed nor would have suggested such a production process for a substrate with a chain molecule immobilized thereon, as in the present claims, this process including the method according to claim 1 or 19 (see claims 17 and 25).

In addition, it is respectfully submitted that the teachings of the applied documents would have neither disclosed nor would have suggested such molecular detection method as in the present claims, having features as discussed previously in connection with claims 1 and 19, and, additionally, wherein the chain molecule is an uprightly disposed single strand molecule (note claim 2), especially wherein the uprightly disposed single strand molecule is a molecule selected from the specific group of substances as in claim 3; and/or wherein the chain molecule immobilized on the substrate surface is a multiple strand molecule comprising an uprightly disposed single strand molecule (comprising nucleic acid) and at least one chain molecule that

can bind to the single strand molecule (can bind to the nucleic acid), as in claims 4 and 21; and/or wherein the multiple strand molecule is a complex of at least one molecule selected from a specific group thereof as in claims 5 and 22; and/or wherein the individual chain molecule, as immobilized, is uprightly disposed relative to the substrate surface so as to extend substantially perpendicularly from the substrate surface (see claims 27 and 28); and/or the more specific definition of the substrate having chain molecules immobilized on the surface thereof, as in claims 31 and 32.

As discussed in more detail infra, it is respectfully submitted that Peeters does not disclose, nor would have suggested, probing with a scanning probe microscope in a solution which is an aqueous solution containing a salt or a buffer solution, as in claim 1 and all claims dependent thereon, and as in claim 35.

In addition, in connection with rejection of claims under 35 USC 103, Henderson-2, et al. relates to removing at least one object from the substrate, while Liu, et al. relates to producing nano patterns of thiolated single-stranded DNA by using an atomic force microscopy (AFM)-based lithography technique known as nanografting. It is respectfully submitted that one of ordinary skill in the art concerned with in Liu, et al. would not have looked to the teachings of Henderson-2, et al., relating to removing the object affixed to a substrate.

Furthermore, Obremski, et al. discloses ligand binding assays in which the analyte is detected and quantified, directly or indirectly, on the basis of its specific affinity for a chemically modified solid material, detecting fluorescence emissions from any solvent zone having an analyte capture complex tagged with a fluorescent label. Even taking the teachings of Obremski, et al. together with the teachings of Liu, et al., and even in light of the teachings of Henderson-2, et al., the teachings of

these references would not have disclosed, or suggested, the probing of an individual chain molecule immobilized on a plastic substrate surface and as immobilized being uprightly disposed relative to the plastic substrate surface with the probing being performed by a scanning probe microscope in a solution which is an aqueous solution containing a salt or a buffer solution (see claim 1) or wherein the single strand molecule is a nucleic acid (see claim 19).

The present invention relates to a molecular detection method, which can be used to visualize and identify localized chain molecules, and a molecular counting method and molecular localization detection method using such molecular detection method.

In immobilizing, e.g., DNA or, more generally, nucleic acid, among other materials, on a substrate, there are known techniques in which DNA is directly synthesized on a substrate, and in which DNA, that has been synthesized separately, is immobilized on a substrate. In each of these techniques, unless the DNA is uniformly and distributedly (nonlocalized) immobilized on an intended section on the substrate, qualitative and quantitative analytic performance cannot be exhibited. Conventionally, there is no technique for examining, at the molecular level, whether or not, e.g., single strand DNA is uniformly immobilized on a specific area on the substrate, and there has been a desire for development of such a testing technique.

As described in the paragraph bridging pages 2 and 3 of Applicants' specification, with regard to means for obtaining information on whether or not immobilized molecules are nonlocalized or localized on a substrate, there is an observation using an electron microscope; however, since this observation is carried out under vacuum, in the case of biopolymers the structure thereof will be destroyed

and observation is not possible, or they stick to the substrate, thus making it impossible to distinguish them from the substrate.

Thus, there is a desire for a molecular detection technique that enables, in a substrate such as a DNA chip or a DNA microarray in which a large number of chain molecules are immobilized, the individual molecules to be visualized and counted while maintaining activity of the chain molecules; and, moreover, wherein information about localization of the molecules can be obtained.

Against this background, Applicants provide a technique achieving the objects referred to in the immediately preceding paragraph. Specifically, Applicants provide a technique wherein an individual chain molecule is visualized and identified easily and accurately, with such individual chain molecule immobilized on a surface of, e.g., a plastic substrate and being immobilized uprightly disposed relative to the plastic substrate surface, the visualizing and identifying being performed by probing with a scanning probe microscope in solution(in particular, an aqueous solution containing a salt or a buffer solution), so as to observe a profile of the plastic substrate surface having the individual chain molecules immobilized thereon and observe the individual chain molecule immobilized uprightly on the plastic substrate surface.

According to the present invention, a DNA or a protein immobilized on a plastic substrate (e.g., a chip or a microarray) can be detected by measuring fluorescence intensity. Compare, for example, with Obremski, et al., detecting fluorescence emission from a zone having an analyte capture complex tagged with a fluorescent label.

By the present invention, using a plastic substrate, a relatively inexpensive substrate is utilized; and, moreover, the individual chain molecules immobilized on the plastic substrate surface, and uprightly disposed relative thereto while

immobilized, can be detected easily and accurately, using a scanning probe microscope and observing a profile of the substrate surface having the individual chain molecules immobilized thereon, and the molecules can be identified, with information about localization obtained.

Moreover, since the molecules can be individually recognized, as compared with conventional detection methods wherein an array is visualized, detection is possible with a small amount of sample, and a high sensitivity detection method is achieved. Note, for example, the paragraph bridging pages 25 and 26 of Applicants' specification.

Furthermore, since the scanning probe microscope is used so as to observe a profile of the plastic substrate surface having individual chain molecules immobilized thereon, the present method can be utilized to visualize, e.g., DNA-protein complexes formed by the pairing of RecA-coated complementary short single-stranded DNA probes with linear double-stranded DNA targets, and can identify the sequence-specific site complementary to the single-stranded DNA probe on the double-stranded DNA target.

Peeters discloses a bionanotechnology method for rapidly determining the surface topology of protein molecules. The method is for discovering protein adsorption sites on a surface, and comprises providing a test surface having a surface topology comprised of a random distribution of randomly shaped features of a size from about 10^{-10} meters (1 Angstrom) to about 10^{-8} meters (10 nanometers) in width, height, depth and spacing; exposing the test surface to a solution of a substantially purified protein, the solution remaining in contact with the surface sufficiently long to enable protein molecules to adsorb to adsorption sites; removing the solution with unadsorbed protein molecules from the test surface; and identifying

the protein adsorption sites by detecting the presence of adsorbed protein molecules, to locate protein molecules adsorbed to the test surface. See column 2, lines 54-67. Note also column 3, lines 18-26, describing another related method that includes detecting the presence of adsorbed protein molecules using a microcantilever; or wherein the presence of adsorbed protein molecules are detected using, for example, various different types of microscopes, or coupling a microparticle to protein molecules adsorbed to the test surface. See also Fig. 3 and the discussion in connection therewith in column 10, lines 46-59.

Initially, it is emphasized that Peeters removes the solution with unadsorbed protein molecules from the test surface. It is respectfully submitted that this reference would have neither disclosed nor would have suggested, and in fact would have taught away from, probing with the scanning probe microscope in the solution, particularly wherein such solution is an aqueous solution containing a salt, or a buffer solution.

Liu, et al. discloses three atomic force microscopy (AFM)-based lithography techniques for creating nanopatterns of self-assembled monolayers (SAMs) and biosensors: nanoshaving, nanografting and nanopen reader and writer (NPRW). This article goes on to disclose that using these techniques, nanostructures of thiols as small as 2 x 4 nm^2 have been successfully produced with various chain lengths and terminal groups; that high-resolution images show that the thiol molecules within the nanopatterns are closely packed; and that in addition to SAM structures, biomolecules such as proteins can be positioned on a surface via selective immobilization. Note the paragraph bridging the left- and right-hand columns on page 863, as well as the sole full paragraph in the right-hand column on page 863. Note also the last full paragraph in the left-hand column on page 864, as well as

Fig. 2 on page 864. The Abstract of Liu, et al. discloses that nanopatterns of thiolated single-stranded DNA (ssDNA) are produced by using the AFM-based lithography technique; the ssDNA molecules adsorb chemically onto an exposed gold area through a sulfur headgroup, and the ssDNA molecules stand up on the gold surfaces and adapt a stretched conformation.

According to the present invention, the chain molecules are individually observed, in order to visualize and identify the individual chain molecules. However, in contrast, in Lui, et al., if the single-stranded DNA molecules were individually observed, nanopatterns consisting of the molecules could not be obtained. In view thereof, it is respectfully submitted that one of ordinary skill in the art involved with in Lui, et al. would not observe the molecules individually so as to produce the nanopatterns consisting of single-stranded DNA molecules.

More importantly, Liu, et al. is concerned with a nanopattern forming technique, attempting to produce the nanopatterns of single-stranded DNA. When producing the nanopatterns of the single-stranded DNA molecules, one of ordinary skill in the art need not visualize an individual single-stranded DNA molecule. Furthermore, if one of ordinary skill in the art visualized the individual single-stranded DNA molecule within the nanopattern, then one could not observe the entire structure of the nanopattern (e.g., square, rectangular or linear). Thus, it is respectfully submitted that this reference would have neither disclosed nor would have suggested such a molecular detection method as in the present claims, including the visualizing and identifying of an individual chain molecule immobilized on a plastic substrate surface and (while immobilized) uprightly disposed relative to the substrate surface, with the visualizing and identifying being performed by probing with a scanning probe microscope in solution, so as to make the observation of the

profile of the substrate surface having the individual chain molecules thereon and observe the individual chain molecule immobilized uprightly on the plastic substrate surface, as in the present claims, or other features of the present invention as discussed previously, and advantages thereof. In this regard, it is emphasized that Liu, et al. discloses visualizing and identifying the aggregates of the thiolated ssDNA molecules produced by using the alkanethiol SAMs.

It is respectfully submitted that the secondary references applied by the Examiner together with Liu, et al. would not have rectified the deficiencies thereof, such that the presently claimed invention as a whole would have been obvious to one of ordinary skill in the art.

Obremski, et al. discloses ligand binding assays in which an analyte is detected and quantified, directly or indirectly, on the basis of its specific affinity for a chemically modified solid material. Note paragraph [0002] on page 1 of this patent document. In the method described in this patent document, an array of sorbent zones is immobilized on a substrate, the sorbent zones including an analyte binding partner, which can be an oligonucleotide probe, antibody, or receptor molecule. When a defined volume of sample, believed to contain an analyte, is deposited on a sorbent zone, the analyte is substantially depleted from the sample to form an analyte capture complex with the analyte binding partner. In one embodiment disclosed in this patent document, the sorbent zones also contain a first binding partner attached to the substrate, wherein the first binding partner forms a first binding complex with a conjugate, the conjugate comprising a first ligand and the analyte binding partner, the first ligand binding specifically with the first binding partner and the analyte binding partner can bind specifically with the analyte. Note paragraphs [0009]-[0011] on page 1 of this patent document. See also

paragraph [0015] on page 2 of this patent document, disclosing preferred substrates selected from the group consisting of polycarbonate, polystyrene, polyethylene, polypropylene and polymethylmethacrylate.

Even assuming, arguendo, that the teachings of Obremski, et al. were properly combinable with the teachings of Liu, et al., such combined teachings would have neither disclosed nor would have suggested the presently claimed invention, including visualizing and identifying an individual chain molecule, immobilized on the substrate (and, while immobilized, being uprightly disposed relative to the substrate), by probing with a scanning probe microscope in solution (e.g., which is an aqueous solution containing a salt or a buffer solution) so as to observe a profile of the surface of the substrate having the individual chain molecules immobilized thereon and observe the individual chain molecule immobilized uprightly on the plastic substrate surface, especially wherein the chain molecule is a nucleic acid; or the other features of the present invention as discussed previously, and advantages thereof.

Henderson-2, et al. discloses a method for the differentiation and evaluation of objects by their binding affinity. According to the described method, a force transduction device is used to differentially separate and/or remove objects that are bound to a surface by regulating an applied force. By incrementally increasing the applied force, one can determine the strength of binding between the surface and the objects on the surface. The objects may include, but are not limited to, molecules, viruses, cells, phage, and other organic or inorganic molecules. Specifically, this patent document discloses a method for determining the binding affinity between an object and a surface, comprising affixing at least one object to a surface, scanning the surface with a scanning probe microscope for locating at least

one object affixed to the surface, applying a force to at least one object on the surface to remove at least one object that has a relatively low binding affinity to the surface, monitoring the force applied to at least one object, and calculating the binding affinity between at least one object and the surface from the force applied to remove at least one object. Note, in particular, paragraphs [0021] and [0024] on page 2 of this patent document. See also paragraph [0029] on page 3 of this patent document.

It is emphasized that Henderson-2, et al. discloses removal of objects, for determining binding affinities. It is respectfully submitted that one of ordinary skill in the art concerned with in Liu, et al., producing nanostructures of DNA on surfaces, would not have looked to the teachings of Henderson-2, et al.

As Liu, et al., is concerned with aggregates, it is respectfully submitted that looking to individual chain molecules, based on the Examiner's characterization of Henderson-2, et al., would destroy Liu, et al. for its intended purpose. Thus, this modification of Liu, et al. is improper. See In re Ratti, 123 USPQ 349 (CCPA 1959). Moreover, Applicants respectfully traverse the Examiner's characterization of the teachings of Henderson-2, et al., again emphasizing that this reference is directed to removal of objects from a surface using a probe.

The contention by the Examiner in the third full paragraph on page 13 of the Office Action mailed July 6, 2010, that Henderson-2, et al. shows that visualizing and identifying an individual chain molecule by scanning probe microscope was known at the time of the presently claimed invention, is respectfully traversed. It is emphasized that Henderson-2, et al. discloses removal of an object from the surface; and it is respectfully submitted that the teachings of this reference would not have properly been combinable with the teachings of, inter alia, Liu, et al., as discussed

previously; but even if properly combinable, and even together with the teachings of Obremski, et al., would have neither taught nor would have suggested the visualizing and identifying as in the present claims, by probing with a scanning probe microscope in a solution as in the present claims, especially wherein the individual chain molecule is visualized and identified, and also especially wherein such individual chain molecule is a nucleic acid.

In view of the foregoing comments and amendments, and, moreover, in view of the concurrently filed RCE Transmittal, entry of the present amendments, and reconsideration and allowance of all claims being considered on the merits in the above-identified application, are respectfully requested.

To the extent necessary, Applicants petition for an extension of time under 37 CFR 1.136. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to the Deposit Account of Antonelli, Terry, Stout & Kraus, LLP, Deposit Account No. 01-2135 (case 1204.45527X00) and please credit any excess fees to such Deposit Account.

Respectfully submitted,

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